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Developmental response to low diets by giant *Nephila clavipes* females (Araneae: Nephilidae)

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Abstract. Female-biased sexual size dimorphism is common in arthropods, apparently driven by fecundity selection in females. Selective pressures that limit growth are less often considered. One factor that researchers have rarely considered is the possible role of energetic limits on growth. The orb weaving spider *Nephila clavipes* (Linnaeus 1767) is extremely sexually size dimorphic. Males are "normal" sized spiders and females are up to ten times longer, having passed through several additional juvenile instars. This extreme size dimorphism presents the opportunity to test for intrinsic energetic costs of gigantism. Prior studies have shown that males successfully reach maturity on a range of diets, while female dietary requirements increase rapidly with increasing size. We here examine the effects of variation in food availability on juvenile female development by randomly assigning spiderlings from six different families (from six distinct populations) to quantitatively varying but qualitatively identical diets. Based upon field observations, we expected that dietary restrictions would have the greatest effect on duration of instars, particularly later instars, and on instar number (because longer total development would lead to curtailment of growth at an earlier stage), with relatively little effect on growth per molt. Because the diets ranged from higher than mean intake observed in the field to well below mean intake, we expected females to mature at a wide range of instars (and sizes). Our results support the functional relationship among food intake, instar duration, and fixed growth per molt (although growth per molt was less canalized than suggested by field observations). However, we observed no variation in number of instars, and we suggest that these data provide additional support for the importance of rare, large prey in the diets of web-building spiders.

Keywords: Phenotypic plasticity, rare large prey hypothesis, survivorship

"Reverse" sexual size dimorphism (SSD) is generally believed due to fecundity selection (Darwin 1871); female arthropods commonly show size-dependent fecundity, where larger or heavier females lay many more eggs (e.g., Miyashita 1986; Higgins 1992a, 2002; Legrand & Morse 2000; Uhl et al. 2004; Fernandez-Montraveta & Moya-Larano 2007). But there must be countervailing selection against continued increases in female size. Proposed selective pressures against larger size include selection for early maturation (e.g., Roff 2001; however, see Berner & Blanckenhorn 2007), selection against developmental asynchrony when males are much smaller than females (Calabrese et al. 2008; L. Higgins & C. Goodnight pers. obs.), and selection imposed by the increased energetic requirements of increased size (Higgins 2002; Higgins & Goodnight 2010).

In *Nephila*, as in most spiders, development is determinant; there are no molts subsequent to the molt to sexual maturity. Age and size at sexual maturity reflect the interaction of juvenile development with the environment (Higgins & Rankin 1996; Berner & Blankenhorn 2007). Size at sexual maturity is determined by growth per molt, which shows little variation in the field (Higgins 1992a, 1993) and the total number of instars (Higgins and Rankin 1996). Age at sexual maturity is determined by the time spent in each instar and the number of instars. *Nephila clavipes* (Linnaeus 1767) females are several instars larger than their male siblings, and juvenile females require accelerating amounts of food, quantities that are an increasing proportion of their mass rather than a constant proportion of their mass (Higgins & Goodnight 2010). Males, maturing in roughly half the number of juvenile stages compared to their female siblings, have a much greater likelihood of reaching maturity under conditions of food stress (L. Higgins & C. Goodnight unpubl. results).

In addition to the energetic constraints that juvenile females may experience, field observations also indicate that females are under different temporal selective regimes than males. The total development time required for females can approach the length of the growing season in many habitats (Higgins 2000), and males develop so much earlier than females that late-maturing females may not be able to find mates (Higgins 1989).

We therefore expect late instar juvenile females to respond very strongly to dietary limitation, and moreover predict, based upon field observations, that instar duration and instar number will vary more than growth per molt. To test our model of developmental responses to diet, we reared *N. clavipes* females on diets that are adequate for young juvenile development and male maturation (Higgins & Rankin 2002; L. Higgins & C. Goodnight pers. obs.). Here we report on the developmental consequences of quantitatively different diets, ranging from relatively poor to more rich than most field observations. The diet treatments were qualitatively identical, consisting of the same prey items in the same proportions. Our analyses include comparison of laboratory growth to field growth and tests for family and dietary influences on juvenile development.

METHODS

We set out to test our predictions by rearing spiders of six Mexican families of the large orb-weaving species *Nephila clavipes* on three quantitatively different diets, all within the range of mean daily prey capture observed in the field. All diets were qualitatively identical and consisted of prey that have been used to rear other spider species successfully, including other *Nephila* (Mayntz et al. 2003; Fernandez-Montraveta & Moya-Larano 2007; N. Ruppel & J. Schneider pers. com.; M. Elgar & L Ceballos pers. com.).

Table 1.—Collecting locations for *Nephila clavipes*. All are in Veracruz except Tolosita, Oaxaca.

Name	Location	Altitude	Seasonality
Nanciyaga	18°27′N, 95°4′W	< 50 m	long wet/warm versus dry/cool
Quihuitztlan	19°40′N, 96°25′W	170 m	long wet/warm versus dry/cool
Fortín de las Flores	18°54′N, 96°60′W	990 m	short wet/warm versus dry/cold
Xalapa	19°30′N, 96°53′W	1000 m	short wet/warm versus dry/cold
Sayula de Alemán	17°52′N, 94°59′W	80 m	long wet/warm versus dry/warm
Tolosita	17°12′N, 95°2′W	50 m	long wet/warm versus dry/warm

***Nephila clavipes* natural history.**—*Nephila* is a relatively small genus of pantropical orb-weaving spiders (Kuntner et al. 2008). Extreme sexual size dimorphism due to the evolution of female gigantism is ancestral to the genus (Kuntner & Coddington 2009). There is a great deal of variation among species in mean size and variation in size (Higgins et al. in press), and male size and female size are evolving independently (Kuntner & Coddington 2009). The source populations for the laboratory experiments are all assumed to be univoltine. Spiders reproduce late in the growing season, females laying up to five egg sacs on or under leaves. All surviving females die with the onset of drought or winter conditions (Higgins 2000), and late females may fail to copulate (Higgins 1989) or may not have time to produce an egg sac (Higgins 2000). The spiderlings hatch and molt within the egg sac, and over-winter as first or second instars. Emergence is triggered in the field by unknown cues, likely a combination of warmth and moisture in most habitats.

Nephila clavipes has determinant growth, and no molts follow maturation. Males in the penultimate instar can be identified due to swelling of their pedipalps, but prior to that point they cannot be distinguished from juvenile females. All but two males in these experiments had entered the penultimate stage by the eighth instar (two delayed to the ninth instar), so we assume that all eighth instar spiders lacking pedipalp swelling are females. The dark, heavily sclerotized epigynal plate near the genital opening indicates female maturity.

We collected mature, gravid females in the fall of 2006 from six Mexican populations (Table 1; voucher specimens housed at the National Museum of Natural History, Smithsonian Institution). These sites span a range of environmental conditions, and represent six populations with low levels of gene flow (J. Nuñez pers. com.). The populations chosen for study fall into three pairs of similar environmental and climate conditions: lowland tropics (Nanciyaga, Quihuitztlan), mid-altitude temperate tropics (Xalapa, Fortín) and lowland seasonally dry tropics (Sayala, Tolosita). Xalapa and Fortín are always univoltine (Higgins 2000; P. Berea pers. com.) and Nanciyaga is usually univoltine (Higgins 2000). Sayala and Tolosita we believe to be univoltine due to the climate similarity between these sites and Chamela, which is univoltine (Higgins 2000; www.tutiempo.net/clima/ accessed 5 March 2009).

Spider rearing.—Mature females collected in the late summer were maintained in the laboratory (ca 75% RH, 10:14 L:D, ca 27° C) on a diet of crickets (fed dog food and apples) and houseflies (from SpiderPharm.com; maintained after maturity on sugar and dry milk with water available from a sponge). We kept the egg sacs laid by these females under the same conditions until hatching and first molt (which happens inside the egg sac). They were then moved to "winter" conditions (10:14 L:D, 4° C for the temperate populations or 16° C for lowland populations; humidity maintained by damp toweling that was checked bi-weekly) for 5–10 weeks to stagger emergence of spiderlings. Due to the logistical difficulties of individually feeding spiderlings on controlled diets, only spiders from one haphazardly chosen egg sac from each population were included in these experiments.

When we were ready to add additional spiders to the experiment, we moved an egg sac into warm conditions (Percival incubator, 75% RH, 14:10 L:D, 27° C). Upon the spiders' emergence and molting to the third instar [leg 1 tibia + patella (TPL) ca 0.1 cm], we placed spiderlings into individual boxes (11 cm wide × 11 cm high × 4 cm deep) with 2.5 cm or 5.1 cm chicken wire for web supports and randomly assigned to a diet treatment (Table 2). We increased food levels by 50% when spiders molted to the sixth instar because prior results indicated that juvenile dietary requirements increase greatly about this stage of development (Higgins & Goodnight 2010).

As the spiders grew, we moved them into larger boxes to accommodate their larger webs: once when they molted to TPL ≥ 0.3 cm (22 cm wide × 10 cm high × 10 cm deep) and again when they molted to TPL ≥ 0.7 cm (31 cm wide × 23.5 cm high × 11 cm deep, Pioneer plastics). All but three males reached sexual maturity prior to TPL = 0.6 cm. To mimic declining day length in natural habitats, all spiders were moved to short-day conditions (11:13h L:D) in a walk-in chamber approximately 100 days after starting the experimental treatment (101 ± 2 days). Most males were sexually mature at the time of the move. Temperature and humidity in the walk-in chamber were less exactly controlled, but averaged 24° C and 72% RH.

We fed all spiders twice weekly, at which time we recorded and removed all uneaten dead flies and recorded if the spider had molted. We recorded size as leg 1 tibia-patella length (TPL) because this is easily and reliably measured without

Table 2.—Feeding regimes for spiders. Weekly food levels as percent of spider mass. * Diet determined individually based on post-molt size at each instar. † Size ranges overlap because spiders on lower diets sometimes grew less per molt, and qualitative diet shifts were established by instar not by size.

Instar(s)	Size range (TPL, cm)†	Quantity of food as percent spider mass			Diet quality (ratio by mass)
		L	M	H	
3–5	0.1–0.45	35	56	84	*D. melanogaster*
6*	0.46–0.80	56	84	126	*D. melanogaster*:*D. virilis* (4:6)
7*	0.70–0.93	56	84	126	*D. virilis*:houseflies (3:7)
8*	0.8–1.2	56	84	126	*D. virilis*:houseflies (2:8)
9* and subsequent	0.9–1.4	56	84	126	*D. virilis*:houseflies (1:9)

removing spiders from their webs (Higgins 1992a). In addition to measuring the spiders using Helios ® needle-nosed calipers (TPL and abdomen length and abdomen width), we retrieved the shed exoskeleton, which served as a physical record of TPL of the prior instar. From abdomen length and width, we calculated abdominal volume as a cylinder and then used this volume and TPL to estimate spider mass after each molt [Higgins 1992a: mass (mg) = 12 + 81 (TPL cm^3) + 784 (abdomen volume cc)]. The size of hard portions of the exoskeleton does not change between molts and serves as a measure of size at each instar.

Weekly food availability (number of prey) was calculated based upon the mean mass of each prey type: *D. melanogaster* (mean mass 0.748 mg, SD = 0.110, n = 11), *D. virilis* (mean mass 1.60 mg, SD = 0.239, n = 15). Since the addition of high-protein dog-food to fly media increases the protein content of the prey and the survival of the spiders (Mayntz et al. 2003), all prey except *D. virilis* were reared on protein-supplemented diets. (*D. virilis* cultures grow slowly, and the addition of dog food to the media resulted in a high frequency of mold overgrowth, killing the culture.) At the seventh instar, we added commercially reared high-protein house flies to the diets (*Musca domestica*, SpiderPharm Inc; mean mass = 11.65 mg, SD = 2.077, n = 10). The qualitative shifts were necessary for logistical reasons. If we had fed only *D. melanogaster* through the entire development, the number of flies provided in later instars would have numbered in the hundreds per week due to the large size of the juvenile females. Within an instar, diet varied quantitatively, but not qualitatively, across treatment groups (Table 2).

Laboratory versus field growth.—In addition to testing for a priori effects of diet and family on juvenile developmental trajectories in the laboratory, we also tested whether juvenile growth in the laboratory was statistically distinct from juvenile growth in the field. Published records of growth per molt and intermolt duration serve as benchmarks (Higgins 1992a, 1993). Because the source populations for this laboratory experiment are not identical to those studied in the field, we took the mean slope of the growth per molt (Higgins 1993: Table 3) as the benchmark. The Mexican field studies did not include measurement of intermolt duration, but prior studies produced no detectable differences among sites as different as Texas and Panama, so we used regression of intermolt duration against spider size Higgins (1992a: Fig. 2) as the benchmark for intermolt duration for the laboratory.

We calculated an expected value of size as a function of premolt size and intermolt duration as a function of current spider size. We calculated expected spider size as $TPL_{postmolt}$ = 0.0567 + 1.263* $TPL_{premolt}$. We calculated expected instar duration as days intermolt = 7.18 + 8.56 (TPL). We then subtracted the expected from the observed and examined the distribution of the residuals as functions of family, diet, and instar.

Field censuses of prey capture.—In 1989–1990, LH worked in seven Mexican sites, including two of the sites from which these spiders were collected (Higgins 2000): Nanciyaga and Fortín de las Flores. The field studies included trap-line censuses of prey-capture success (Higgins & Buskirk 1992; Higgins 1993). The published analyses consider only median prey size and mean prey capture rates. To describe the foraging success of spiders in nature more fully, we here present an analysis of size distribution of prey, total prey mass captured as a function of spider size, and the likelihood of capture of different amounts of prey by spiders larger than TPL = 0.5 cm across all seven populations.

Size, age and instar at sexual maturity.—Female *N. clavipes* have heavily sclerotized epigynal plates allowing us to recognize when an individual molted to sexual maturity. We compared age, size, and instar at sexual maturity across all mature females on all diets to test for significant variation due to family. We compared across diets to test whether spiders with reduced diets take longer in each instar and therefore mature at a greater age (in days) but earlier instar (smaller size) relative to those on higher diets, thus avoiding end-of-season penalties detected in prior field studies (Higgins 2000).

Statistical analyses.—All statistical analyses were done in JMP (Version 7.0.2). Preliminary analyses of the distribution of age (time since initiation of treatment), instar duration and size (TPL) indicated that natural log transformation was necessary for normal distribution of developmental data; size

Table 3.—ANOVAs of female size and age at the ninth instar (log transformed).

	Source	*df*	SS	*F*	*P*
Size (ln TPL)					
	family	5	0.1478	2.6543	0.030
	diet	2	0.5960	26.76	< 0.0001
	family * diet	10	0.2376	2.133	0.033
	error	70	0.7797		
Age (ln days)					
	family	5	0.4158	5.223	0.0004
	diet	2	1.686	52.96	< 0.0001
	family * diet	10	0.3180	1.997	0.0465
	error	70	1.1144		

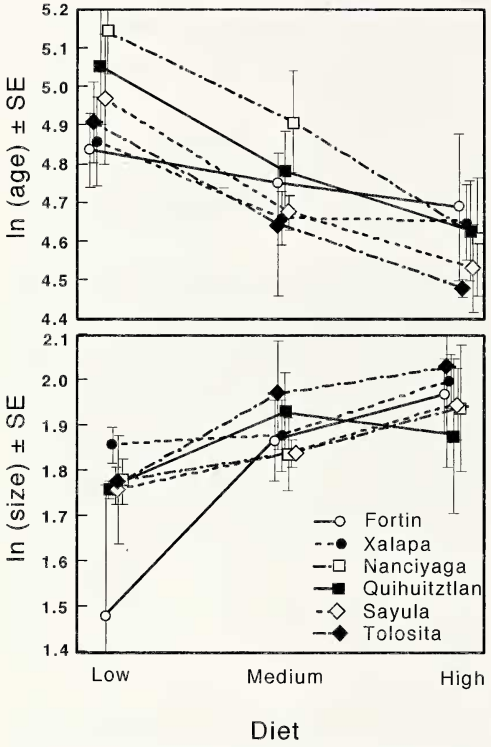

Figure 1.—Norm of reaction response to diet by age (days) and size (TPL) at which females reach the ninth instar (six instars of experimental treatment). Fortín females are significantly different from the other populations, driving a significant population x diet interaction.

data were transformed to mm prior to log-transformation. However, in the comparison of field and laboratory data, we found that the residuals of observed minus expected were normally distributed and hence required no transformation.

RESULTS

Developmental response to diet.—A total of 190 spiders molted to the fourth instar, and most spiders survived to the ninth instar (at which time all but three males had reached sexual maturity), allowing us to test for diet effects on development in known females (discarding data from individuals that died prior to the eighth instar). The diets significantly altered developmental trajectories of older juvenile females.

If, as we suspect, juvenile females are accelerating their growth rates in later instars to both reduce developmental asynchrony with males and to reduce risk of maturing late relative to the end of the season (Higgins 2000), we expected to find that spiders on low diets sacrifice growth per molt to pass through instars more quickly or to shorten their development by reducing the number of juvenile instars. We tested these predictions separately.

To test for cumulative changes in intermolt duration and growth per molt among spiders reared on different diets, we ran a MANOVA (multivariate analysis of variance) testing developmental differences in age and size at the ninth instar with diet and family as independent variables. We ran this analysis using data from spiders in the ninth instar (six experimental instars) because of high female mortality on low and medium food levels after this stage and because at this stage all males were identified and could be excluded. A total of 88 females reached the ninth instar. The fully-factoral MANOVA of ln (TPL) and ln (total time to ninth instar) was highly significant for both factors and the interaction (Fig. 3, whole model: Roy's Maximum Root (RMR) = 3.53, approximate $F = 14.51$, $df = 17$, $P < 0.001$; family: RMR = 0.458, $df = 5$, $P < 0.001$; diet: RMR = 2.752, $df = 2$, $P < 0.001$; family * diet: RMR = 0.353, $df = 10$, $P = 0.0134$).

Spiders on lower diets were smaller and reached the ninth instar later than spiders on the high diets. Examination of the E and H matrices showed that spiders from some families took longer to reach the ninth instar and were smaller when they did. Most interestingly, the interaction of family and diet reflects the fact that spiders from families that took less time to reach the ninth instar were more uniform in size across diets. Examination of the norm-of-reaction curves for each developmental parameter across all families (ANOVA, Table 3) shows that Fortín responded to diet with less change in time and great difference in size at the ninth instar (Fig. 1), and spiders from this family are presumably responsible for the significant interaction effects. We emphasize that because we only used one egg sac per family, we cannot know whether the unique developmental response by Fortín females represents a general characteristic of this population, or instead occurred because the particular family from Fortín was unusual.

The analysis of females at one single instar obscures changes that may take place as the spiders develop. To test for differences among families in the developmental responses to diet, we ran separate fully factorial ANCOVA with instar number (developmental stage) as the covariate, testing for developmental changes in instar duration and growth in TPL at each molt. As discussed in depth elsewhere (L. Higgins & C. Goodnight in prep.), we recognize that these analyses violate the assumption of independence of measures, since instar should be treated as a nested factor within individual. However, if instar is nested within individual, we cannot test for developmental effects of family and diet (detected as interaction between instar and the factor of interest), because each individual has only one family and diet assignment. Repeated measures ANOVA is also not permitted because developmental data are serially correlated, as demonstrated by the significant effect of instar number, and this violates the assumption of equal correlations between all pairs of observations. We therefore use instar as an independent cofactor as a compromise analysis.

Instar duration increases in later instars and is generally longer in spiders fed lower diets (Fig. 3). However, families varied significantly in their response to diet (family*diet) and in the rate at which instar duration increased (family*instar) (Table 4a). The rate of increase in instar duration was reduced

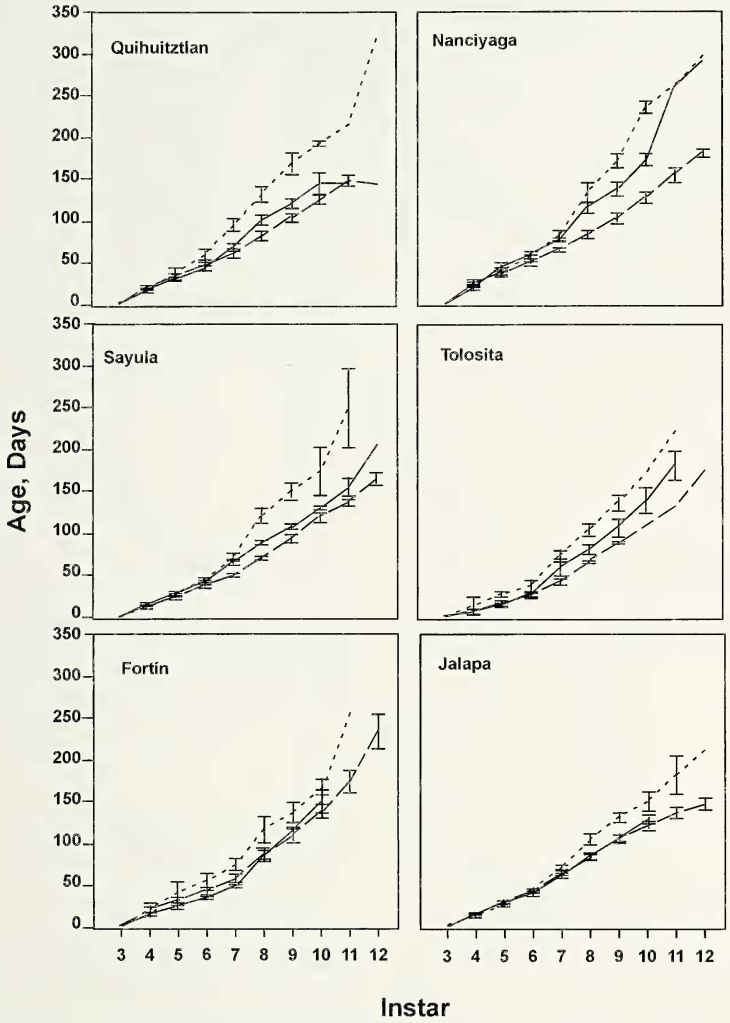

Figure 2.—Mean age (days, ± SE) of females at the molt to each instar, by diet (long dash: high; solid line: medium; short dash: low). Absence of error bar indicates a single surviving individual.

in well-fed spiders: spiders on low diets showed the greatest increase in instar duration as they passed through successive instars (diet*instar in Table 4a). The families did not differ significantly in how instar duration interacted with diet over the developmental trajectory (insignificant three-way interaction). To better understand how instar duration changes over development within each family, we present the regression equation for each family–diet combination in Table 5.

Figure 3 shows that these females increased in size with successive molts on all diets (compared to some arthropods where reduced diets result in molts without growth or even negative growth: Higgins and Rankin 1996). Compared to instar duration, the change in size at each molt responded less to the different experimental variables (Table 4b). Over all families, the change in TPL at each molt [calculated as ln (premolt TPL - postmolt TPL, cm)] increased slightly but

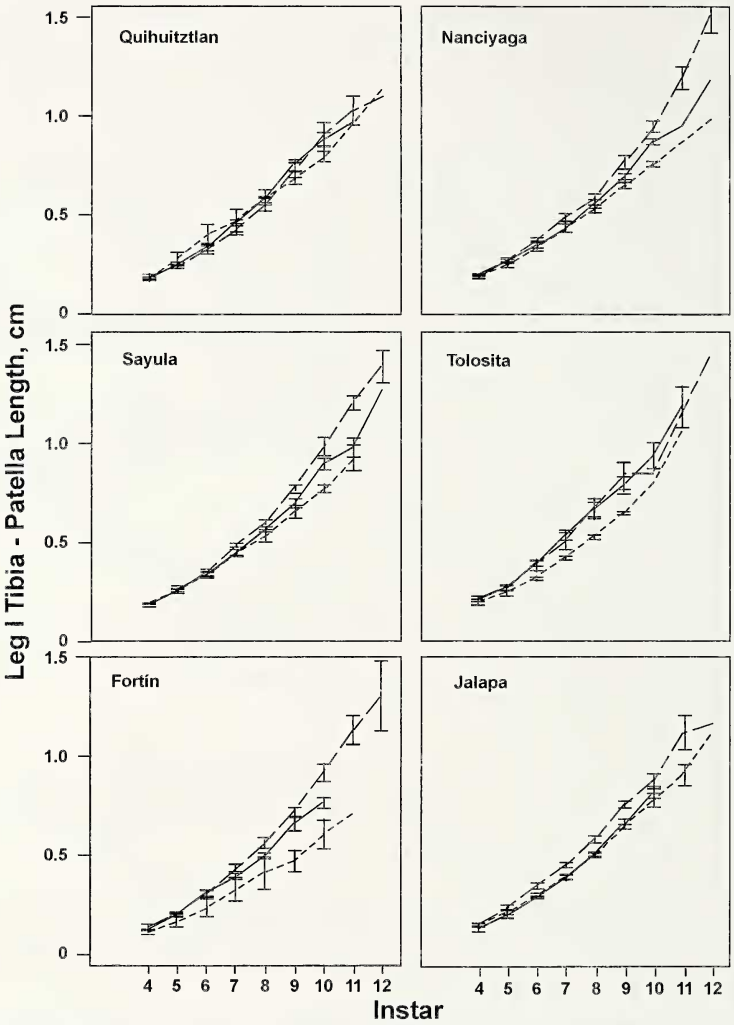

Figure 3.—Mean size (TPL, cm, ± SE) of females in each instar, by diet (long dash: high; solid line: medium; short dash: low). Absence of error bar indicates a single surviving individual.

significantly with successive molts. The rate of increase was unaffected by diet (no instar * diet effects). The significant effect of diet reflects differences in the intercept of the regression lines. Across all instars, diets, and families, growth per molt averaged 0.126 cm (n = 584 molts, SD = 0.062).

Growth in the laboratory versus in the field.—Extensive field data exist that describe growth of *N. clavipes* in Panama, various populations in Mexico, and Texas (Higgins 1992a, 1993). We used these observations to test whether the laboratory conditions produced normal development. ANCOVA showed that spiders were smaller at each instar compared to the field, and this difference increased as spiders grew (significant effect of instar: Table 6a). The deviation from field observations was less in the spiders fed high diets than those fed medium or low diets [diet effect: Student's t-test of LS mean differences = 1.96, P = 0.05; mean (SE)

Table 4.—ANCOVA of development across instars as a function of family and diet.

a. Instar duration (days, log transformed).

Source	*df*	SS	*F*	*P*
family	5	1.015	1.392	0.225
diet	2	16.01	54.92	< 0.0001
family*diet	10	3.021	2.072	0.0250
instar	1	51.09	350.5	< 0.0001
family*instar	5	3.411	4.680	0.0003
diet*instar	2	0.908	3.114	0.045
family*diet*instar	10	2.231	1.530	0.125
error	553	80.61		

b. Change in size at each molt (log transformed)

Source	*df*	SS	*F*	*P*
family	5	1.238	1.82	0.107
diet	2	7.652	28.16	< 0.0001
family*diet	10	1.553	1.143	0.327
instar	1	40.11	295.3	< 0.0001
family*instar	5	0.203	0.3000	0.9137
diet*instar	2	0.764	2.811	0.061
family*diet*instar	10	1.272	0.936	0.500
error	546	74.17		

deviations: high diet = −0.034 (0.003); medium diet = −0.0397 (0.0035); low diet = −0.0469 (0.0040)], but was unaffected by family. The three-way significant interaction of family, diet, and instar reflects that the Fortín spiders fed on low diets deviated more from the field observations than the other families. Similar ANCOVA analysis showed that the duration of each instar was longer in the laboratory compared to the field, the deviation increased as the spiders grew, and was affected by family and diet in complex fashion: all interactions except the three-way interaction were significant (Table 6b).

Table 5.—Instar duration increases in later instars (log transformed days between molts). * $P \leq 0.05$; ** $P < 0.01$ *** $P < 0.001$.

Family/ location	Diet	Intercept	Slope	R^2	*F* (*df*)
Fortín	High	2.095	0.215	0.658	78.97*** (1, 41)
	Med	1.858	0.307	0.646	27.31*** (1, 15)
	Low	2.469	0.165	0.227	4.984* (1, 17)
Xalapa	High	2.667	0.050	0.095	5.142* (1, 49)
	Med	2.393	0.138	0.424	16.21*** (1, 22)
	Low	2.557	0.159	0.426	24.44*** (1, 33)
Nanciyaga	High	2.192	0.158	0.468	50.183*** (1,57)
	Med	2.681	0.131	0.258	8.351 ** (1, 24)
	Low	2.504	0.228	0.525	34.78*** (1, 29)
Quihuitztlan	High	2.402	0.108	0.253	16.90*** (1, 50)
	Med	2.523	0.120	0.240	8.818** (1, 28)
	Low	2.875	0.129	0.233	5.165* (1, 17)
Sayala	High	2.295	0.131	0.391	35.91*** (1, 56)
	Med	2.513	0.114	0.421	21.80*** (1, 30)
	Low	2.391	0.260	0.550	43.93*** (1, 36)
Tolosita	High	1.955	0.223	0.672	30.72*** (1, 15)
	Med	2.119	0.233	0.519	25.85*** (1, 24)
	Low	2.228	0.266	0.592	14.53** (1, 10)

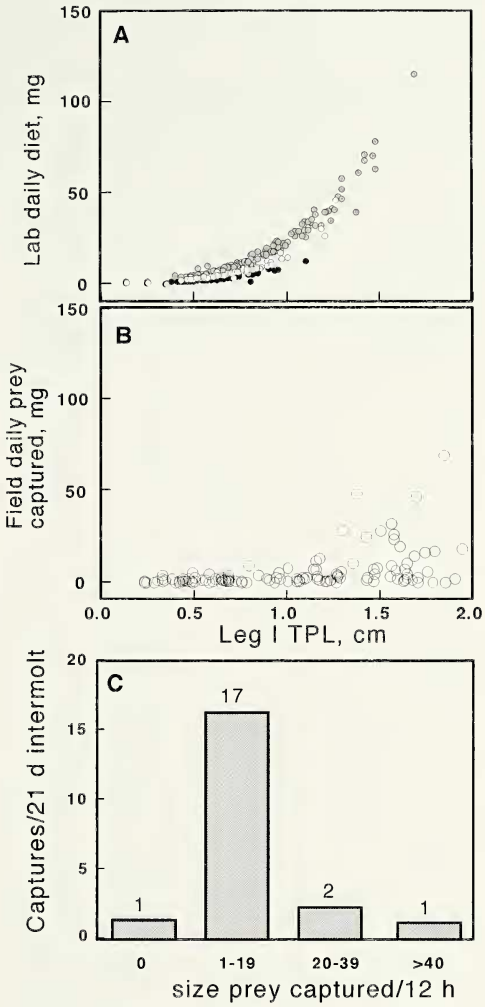

Figure 4.—Total daily prey (A) offered in the laboratory (black – low, white – medium, grey – high) and (B) captured in the field as a function of spider size. (C) For spiders of TPL ≥ 1.0 cm, there is low frequency of capture of large prey in each 21-day instar.

Size and age at sexual maturity.—Since instar duration responded more than growth per molt to diet and family effects, we expected that diet and possibly family would have significant effects on the total number of instars and thus on final size at maturity. Slowly growing spiders on lower diets would curtail their development at an earlier instar. However, we cannot test for family effects because only 25 females

Table 6.—ANCOVA comparisons of residuals of field versus laboratory growth, as measured by a) size and b) duration of *Nephila* instars.

a) Size at each instar as a function of instar number

Source	*df*	SS	*F*	*P*
family	5	0.00545	0.6033	0.697
diet	2	0.0234	6.471	0.002
family*diet	10	0.00827	0.4583	0.9167
instar	1	0.1532	84.86	< 0.0001
family*instar	5	0.00391	0.4339	0.8250
diet*instar	2	0.00507	1.406	0.2459
family*diet*instar	10	0.03665	2.030	0.0286
error	539	0.973		

b) Duration of each instar as a function of instar number.

Source	*df*	SS	*F*	*P*
family	5	0.6167	2.288	0.0451
diet	2	6.328	58.69	< 0.0001
family*diet	10	1.317	2.443	0.0076
instar	1	5.256	97.49	< 0.0001
family*instar	5	1.096	4.066	0.0013
diet*instar	2	0.4279	3.968	0.0196
family*diet*instar	10	0.3897	0.7227	0.703
error	456	24.59		

reached sexual maturity. Survivors are distributed across all family groups. Survival of high-diet individuals would have been greater but the humidifier in the walk-in chamber ceased running for nearly a week, and the resultant low humidity resulted in 75% mortality of the high-diet Xalapa females and nearly 40% mortality of the high- and medium-diet Quihuitzlan females. Interestingly, most of the small juveniles on low diets in the room survived, suggesting that low humidity, like low food availability, may have a disproportional impact on large juvenile females. With few individuals from each family and only slight developmental differences among families (none in growth/molt), we pooled across family to test for effects of diet on the size and age at maturity.

Both size and age at maturity were significantly altered by diet (MANOVA of log-transformed TPL and age (days since initiation of experiment); Roy's Maximum Root =1.808, approximate $F_{(2, 22)} = 19.89$, $P < 0.001$). A posteriori contrast determined that there was no significant effect of diet in the comparison of spiders fed high or medium food levels. Separate ANOVA confirmed that spiders fed high or medium diets matured significantly earlier ($F_{(2, 22)} = 9.29$, $P = 0.001$) and at a larger size ($F_{(2, 24)} = 7.18$, $P = 0.004$) (Table 7). At least within the limitations of this small sample, the number of juvenile instars was not determined by juvenile diet (ANOVA: $F_{(2, 22)} = 0.185$, $P = 0.83$; a power test shows 8% chance of failure to detect a significant difference). Thus, size variation among dietary groups must be due to the cumulative effects of differences in growth at each molt. Individuals reached sexual maturity in either the 11th or 12th instar, and both developmental pathways were represented in all three diet treatments (a non-independent test for effect of instar number on size at maturity showed no significant difference: $F_{(1, 23)} = 1.3$, $P > 0.3$). Importantly, the size of these mature females falls within but at the lower end of the range observed in the field in Mexico (Higgins 2000).

Realism of diet levels.—Compared to spiders observed in the field, those in the laboratory had longer intermolts, lower growth per molt, and smaller size at maturity. Together, these data suggest that even the high laboratory diets may be lower than the field. However, comparison to the field shows this is not the case. Comparing the mass of prey offered in the laboratory to observations from Mexican field studies, we found that the three feeding regimes for spiders smaller than 0.7 cm TPL were within the range of daily prey capture observed in the field, and the low and medium diets stayed within that range over all sizes of spider (Fig. 4A, B). The highest feeding regime was actually higher than most field observations for larger juveniles.

However, in the field, spiders capturing large masses of prey in a single day (> 50 mg: Fig. 4B) have captured rare, large prey items rather than many small items. Using the field data, we estimated the per-instar likelihood of capture of prey of different sizes using the average instar duration of large juvenile females in the field (21 days; Higgins 1992a). On average, a large juvenile female will only have a 50% chance of capturing one large insect during each instar (Fig. 2C) – in half of the larger instars, spiders fail to capture even one large insect. There is a 1.6% chance that any given spider will fail to capture any prey item larger than 10 mg in any given 21-day instar.

DISCUSSION

Developmental responses to differences in food quality, foraging success, and climate are ubiquitous and diverse among arthropods (reviewed in West-Eberhard 2003). *Nephila clavipes* females reared in the laboratory showed strong developmental responses to juvenile diets experienced, but contrary to our expectations, these responses did not include changes in the number of juvenile instars. Importantly, the size at maturity among these laboratory-reared spiders falls at the lower end of the range observed in the field. We propose that our high dietary treatment is actually the minimum at which females can reach sexual maturity, and that these needs are met in the field by the rare capture of very large insects.

As described by Higgins & Rankin (1996), arthropod post-embryonic development can be described by three parameters: intermolt duration, growth per molt, and number of juvenile instars. Combined with prior field data, our results suggest that in *N. clavipes*, these developmental parameters differ in their plasticity and respond to different aspects of prey capture.

Table 7.—Female size, instar, and age at sexual maturity in each diet group, pooled across families.

Diet	*n*	Mean TPL, cm (SD)	Mean age, days (SD)	Mean instar (SD)
High	18	1.29 (0.1623)	177.6 (29.22)	11.6 (0.502)
Medium	2	1.23 (0.0424)	204 (–)	11.5 (0.707)
Low	4	1.020 (0.0753)	259.3 (53.18)	11.8 (0.500)

Intermolt duration is far more variable than growth per molt in the field and in the laboratory. The experimental data we report here support previous hypotheses concerning the interrelationships between growth per molt and instar duration. Because change in size at each molt is closely correlated with premolt mass (Higgins 1992a), and growth per molt is relatively inflexible (see below and Higgins 1992a, 1993), Higgins (1992a) hypothesized that spiders with low prey-capture success experienced extended intermolt duration as they "waited" to accumulate necessary body mass for the next molt.

We did detect significant effects of diet on growth per molt; the spiders fed the lowest diets in the laboratory did show reduced growth per molt in each instar. In the laboratory, the lowest prey capture rates were at the low end of mean prey capture observed in the field. We suspect that spiders capturing such low amounts of food in the field fail to survive because of increased exposure to predators. Predation is very high on small and medium-sized juveniles (Higgins 1992b), and extending the intermolt period extends this period of vulnerability. Only in the laboratory could we observe plasticity in this developmental parameter. Such plasticity may be considered nonadaptive (sensu Ghalambor et al. 2007) in that it is a physiological response to environmental stress rather than an adaptive response to environmental variation.

As growth per molt varies little in field populations of *N. clavipes* (Higgins 1992a, 1993), the large amounts of variation in adult female size are necessarily due to differences in instar number (Higgins & Rankin 1996). However, we were unable to elicit variation in instar number in this experiment. Therefore, the observed variation in size at maturity among females fed different amounts of prey reflects accumulated differences in the growth per molt. Importantly, maturing females were at the low end of the range of sizes observed in the field. The laboratory mean female TPL of 1.2 cm is roughly one instar smaller than the mean of 1.8 cm TPL and two instars smaller than the largest spiders observed in the lowland tropical populations in Mexico (Higgins 2000). In contrast, males in this experiment matured at sizes that span the range observed in the field (L. Higgins & C. Goodnight pers. obs.). This is consistent with the idea that the variation in female size observed in the field reflects variation not in the baseline prey capture rates experienced by each individual (which is equivalent to food quantities in the laboratory), but rather variation in the rare capture of large insects.

We recognize that very few females reached sexual maturity in the laboratory: only 28% of the spiders that reached the ninth instar successfully matured, reflecting very high mortality in late instars on the low and medium diets (L. Higgins & C. Goodnight pers. obs.). We do not believe that this mortality reflects a qualitative nutritional deficit for several reasons. First, the diets we used fall within the range of mean prey-capture rates observed among the diverse Mexican populations (Higgins 2000) and match the feeding regimes that result in normal intermolt durations for small juveniles (Higgins & Rankin 2001). Second, protein-enhanced flies have been used successfully to rear a wide range of spiders including orb-weavers (e.g., Mayntz et al. 2003; G.W. Uetz pers. com.; C. Kristensen pers. com.) and male *N. clavipes* (L. Higgins & C. Goodnight pers. obs.). We have no reason to believe that juvenile *N. clavipes* females have distinct nutritional requirements from other orb weavers or from males of their own species.

We propose that although our laboratory diets fall within the range of observed mean prey-capture rates, they fail to mimic a different kind of variation, the rare capture of large insects (Venner & Casas 2005). Reanalysis of prior field data shows that capture of large insects is more irregular than previously appreciated. In addition to the mean biomass of prey (reported in Higgins 2000), larger juvenile and mature female spiders have a 50% chance of capturing one exceedingly large prey item per instar. We postulate that the capture of rare large prey is vital for successful female maturation in this species. Thus, variation in adult female size, determined primarily by variation in instar number, reflects variation in the rate of capture of large insects rather than simply variation in mean prey capture rate. As pointed out by Blackledge (2011), this may be a general phenomenon of orb-weaving spiders and requires a different approach to field and laboratory investigations of foraging and diet-dependent development and reproduction.

Based upon this hypothesis, we predict that the largest females in each population are adding juvenile instars (Esperk et al. 2007) and reaching large size because they inhabit good microhabitats, defined as those with high frequency of rare, large insects. Although diet-dependent variation in number of instars has been seen in other spiders [e.g., Mayntz et al. 2003 in the orb-weaver *Zygiella x-notata* (Clerck 1757)], it is by no means universal [e.g., Jespersen & Toft 2003 in the wolf spider *Pardosa prativaga* (L. Koch 1870)]. Some spiders, particularly smaller species, may have canalized their number of juvenile instars (e.g., Uhl et al. 2004). It is noteworthy that none of these species achieve size or mass approaching that of mature female *Nephila*, and that the numbers of juvenile instars are roughly half of that observed for females in the current study.

It has been proposed that most arthropod predators experience food limitation most of the time (Ward & Lubin 1993; Bilde & Toft 1998; Kreiter & Wise 2001). However, these studies involved much smaller species and emphasized female fecundity and the likelihood of reproduction rather than juvenile survival and development. Moreover, in most cases, the females were required to capture only a single large prey in order to reproduce successfully. The increasingly strong response to diet with higher instar number found in the current study may reflect the increasing metabolic needs of these giant females, particularly in populations with short time horizons relative to the total developmental time. An analogous dependence on rare, large prey has been found for penultimate and adult females of other spiders with female gigantism (LeGrand & Morse 2000; Moya-Laraño et al. 2003; Venner & Casas 2005). The fecundity advantage of female gigantism thus may come at the cost of increasing dependence upon repeated success at achieving a rare event, the regular capture of very large prey.

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